Development of a Curated Database of *In Vivo* Estrogenic Activity

P Ceger[1], N Kleinstreuer[1], X Chang[1], B Jones[1], J Hamm[1], L Rinckel[1], D Rotroff[2], J Strickland[1], W Casey[3], D Allen[1]

[1]ILS/NICEATM, RTP, NC, USA; [2]North Carolina State University, Raleigh, NC, USA; [3]NIH/NIEHS/DNTP/NICEATM, RTP, NC, USA

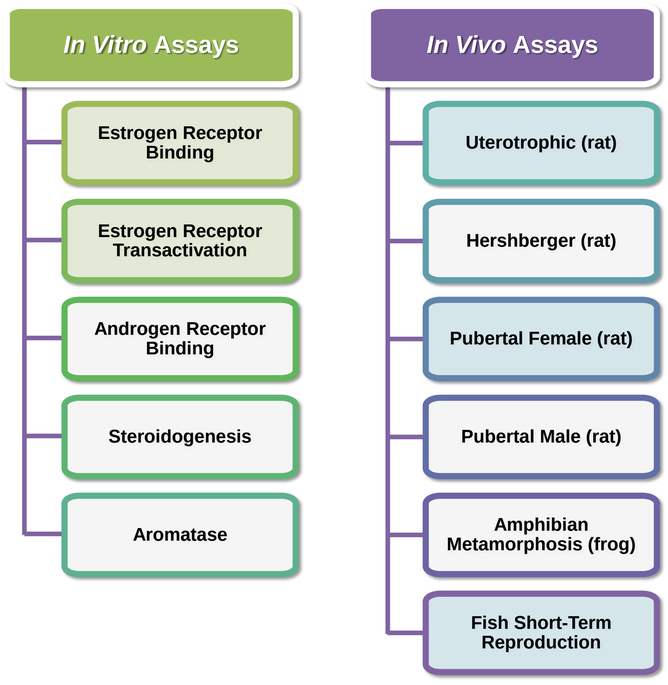
Introduction

- U.S. (7 U.S.C. 136, 110 Stat 1613) and international regulations require the testing of chemicals for potential endocrine activity.
- Approximately 10,000 chemicals lack sufficient testing data, which increases by several hundred new chemicals each year (EPA 2011).
- The U.S. Environmental Protection Agency (EPA) developed a two-tiered strategy to identify endocrine active chemicals (EACs).
- Testing is divided into two tiers, in which Tier 1 consists of *in vitro* and short-term *in vivo* animal tests, and Tier 2 consists of longer-term *in vivo* tests.
- Tier 1 testing (**Figure 1**) may cost millions of dollars per chemical, take years to complete, and utilize many animals.
- High-throughput screening and computational toxicology tools are being developed to identify potential EACs and prioritize further screening efforts.
- A comprehensive, curated database of *in vivo* reference data is needed for successful evaluation, acceptance, and implementation of these tools.
- The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) assembled a comprehensive database of high-quality *in vivo* EAC data to be used to:
  - Link *in vivo* effects to specific pathway perturbations
  - Evaluate the impact of exposure duration on biological responses
  - Evaluate species-specific responses to chemicals
  - Develop and evaluating physiologically-based pharmacokinetic models
  - Validate *in vitro* and *in silico* models of estrogenic activity
  - Prioritize chemicals for further testing

Conclusions

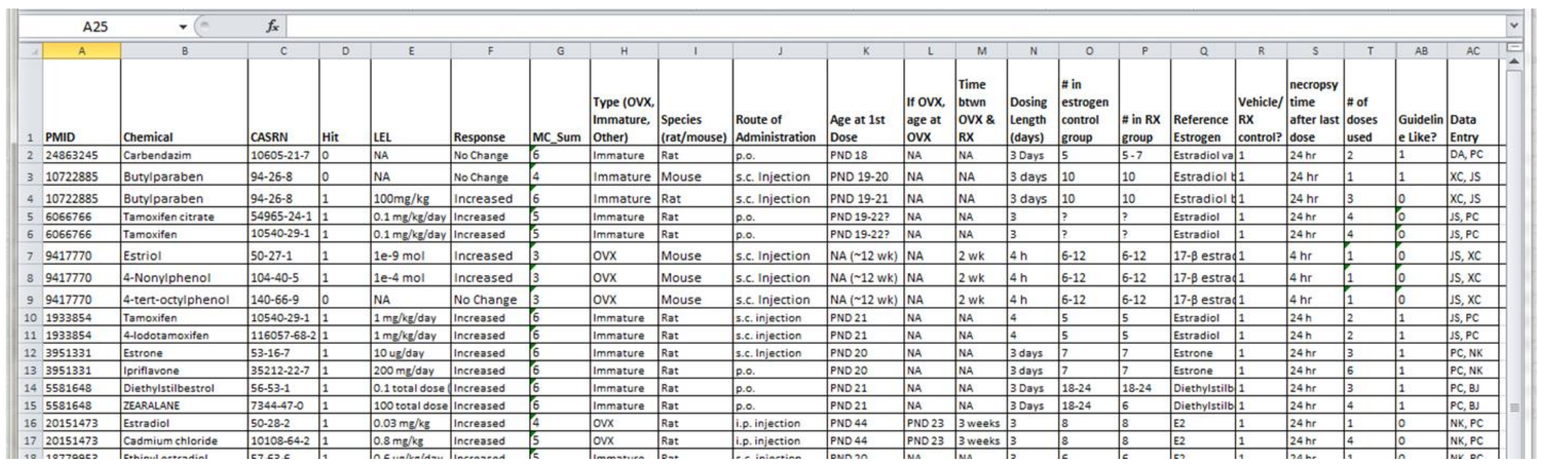
- Regulatory agencies require data on endocrine activity from thousands of chemicals that have not yet been evaluated. Using current methods, this task will take decades to complete and cost millions of dollars.
- High-throughput screens and computational toxicology tools are being developed to identify estrogenic compounds and prioritize further testing.
- NICEATM assembled a comprehensive database of high-quality *in vivo* uterotrophic data that can be used to evaluate high-throughput screens and computational tools for estrogenic activity.
- Quality review of the database is continuing, but at the time of this publication it contained guideline-like uterotrophic studies for 111 ToxCast/E1K chemicals and data from non-guideline studies for another 98 ToxCast/E1K chemicals.
- The database will be made available to the public via the NTP website (http://ntp.niehs.nih.gov/go/40658).

Figure 1. EPA Tier 1 Battery*



*Assays in shaded boxes are capable of detecting estrogenic activity.

Figure 3. Partial Screen Shot of the NICEATM Uterotrophic DB

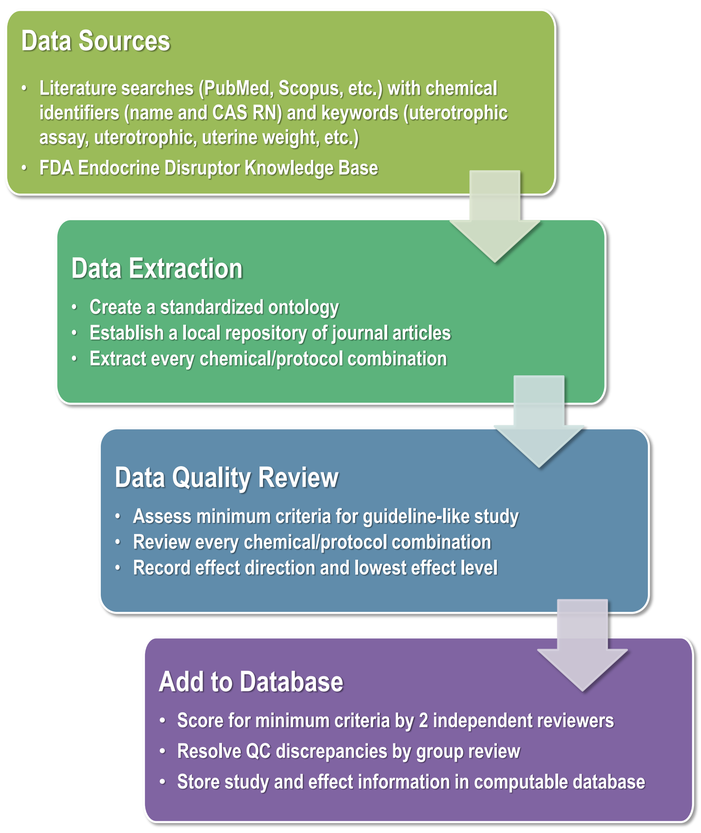


	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	AB	AC
1	PMID	Chemical	CASRN	Hit	LEL	Response	MC_Sum	Type (OVX, Immature, Other)	Species (rat/mouse)	Route of Administration	Age at 1st Dose	If OVX, age at OVX	Time btwn OVX & RX	Dosing Length (days)	# in estrogen control group	# in RX group	Reference Estrogen	Vehicle/ RX control?	necropsy time after last dose	# of doses used	Guideline Like?	Data Entry
2	24863245	Carbendazim	10605-21-7	0	NA	No Change	6	Immature	Rat	p.o.	PND 18	NA	NA	3 Days	5	5 - 7	Estradiol va	1	24 hr	2	1	DA, PC
3	10722885	Butylparaben	94-26-8	0	NA	No Change	4	Immature	Mouse	s.c. Injection	PND 19-20	NA	NA	3 days	10	10	Estradiol b	1	24 hr	1	1	XC, JS
4	10722885	Butylparaben	94-26-8	1	100mg/kg	Increased	6	Immature	Rat	s.c. Injection	PND 19-21	NA	NA	3 days	10	10	Estradiol b	1	24 hr	3	0	XC, JS
5	6066766	Tamoxifen citrate	54965-24-1	1	0.1 mg/kg/day	Increased	5	Immature	Rat	p.o.	PND 19-22?	NA	NA	3	?	?	Estradiol	1	24 hr	4	0	JS, PC
6	6066766	Tamoxifen	10540-29-1	1	0.1 mg/kg/day	Increased	5	Immature	Rat	p.o.	PND 19-22?	NA	NA	3	?	?	Estradiol	1	24 hr	4	0	JS, PC
7	9417770	Estriol	50-27-1	1	1e-9 mol	Increased	3	OVX	Mouse	s.c. Injection	NA (~12 wk)	NA	2 wk	4 h	6-12	6-12	17-β estra	1	4 hr	1	0	JS, XC
8	9417770	4-Nonylphenol	104-40-5	1	1e-4 mol	Increased	3	OVX	Mouse	s.c. Injection	NA (~12 wk)	NA	2 wk	4 h	6-12	6-12	17-β estra	1	4 hr	1	0	JS, XC
9	9417770	4-tert-octylphenol	140-66-9	0	NA	No Change	3	OVX	Mouse	s.c. Injection	NA (~12 wk)	NA	2 wk	4 h	6-12	6-12	17-β estra	1	4 hr	1	0	JS, XC
10	1933854	Tamoxifen	10540-29-1	1	1 mg/kg/day	Increased	6	Immature	Rat	s.c. injection	PND 21	NA	NA	4	5	5	Estradiol	1	24 h	2	1	JS, PC
11	1933854	4-Iodotamoxifen	116057-68-2	1	1 mg/kg/day	Increased	6	Immature	Rat	s.c. injection	PND 21	NA	NA	4	5	5	Estradiol	1	24 h	2	1	JS, PC
12	3951331	Estrone	53-16-7	1	10 ug/day	Increased	6	Immature	Rat	s.c. Injection	PND 20	NA	NA	3 days	7	7	Estrone	1	24 hr	3	1	PC, NK
13	3951331	Ipriflavone	35212-22-7	1	200 mg/day	Increased	6	Immature	Rat	p.o.	PND 20	NA	NA	3 days	7	7	Estrone	1	24 hr	6	1	PC, NK
14	5581648	Diethylstilbestrol	56-53-1	1	0.1 total dose	Increased	6	Immature	Rat	p.o.	PND 21	NA	NA	3 Days	18-24	18-24	Diethylstilb	1	24 hr	3	1	PC, BJ
15	5581648	ZEARALANE	7344-47-0	1	100 total dose	Increased	6	Immature	Rat	p.o.	PND 21	NA	NA	3 Days	18-24	6	Diethylstilb	1	24 hr	4	1	PC, BJ
16	20151473	Estradiol	50-28-2	1	0.03 mg/kg	Increased	4	OVX	Rat	i.p. injection	PND 44	PND 23	3 weeks	3	8	8	E2	1	24 hr	1	0	NK, PC
17	20151473	Cadmium chloride	10108-64-2	1	0.8 mg/kg	Increased	5	OVX	Rat	i.p. injection	PND 44	PND 23	3 weeks	3	8	8	E2	1	24 hr	4	0	NK, PC

Scope of the Database

- EACs may affect the estrogen, androgen, and thyroid systems. This database focuses on estrogenic effects of EACs exhibited by uterine hypertrophy or hypotrophy.
- The literature review initially focused on 1781 chemicals selected by the EPA for the ToxCast (Dix 2007) and E1K screening programs (EPA 2012), all of which were tested in *in vitro* assays that detect changes in endocrine signaling. These chemicals include known negatives and positives with a wide potency range.
- The final database includes data from 160 "guideline-like" studies that adhere to a set of minimum criteria based on EPA and OECD test guidelines for the *in vivo* uterotrophic assay (EPA 2009; OECD 2007) (see **Data Review**). The database also contains data from over 600 "non-guideline" studies that included collection of uterine weight but that did not meet one or more of the minimum criteria for "guideline-like" studies.
- **Figure 2** outlines the process of the literature review and database development.

Figure 2. Data Collection Overview



Abbreviations: CAS RN = Chemical Abstract Service Registry Number; FDA = U.S. Food and Drug Administration; QC = quality control.

Review of the Literature

- PubMed, Scopus, and EmBase™ databases were queried through multiple searches using:
  - **Substance name, known synonyms, and Chemical Abstract Service Registry Number (CAS RN):** synonyms and CAS RNs were obtained from the ChemID Plus website (National Library of Medicine 2013).
  - **Keywords including:** "uterotrophic assay", "uterotrophic", "uterotropic" (as an alternate spelling of "uterotrophic"), "uterine weight", and "uterus"
- The PubMed search process was simplified by using PubMatrix, an open source tool for multiplex literature searches (Becker 2003). **Table 1** lists results from an example PubMatrix search.

Table 1. Example PubMatrix Search

Chemical ID	Uterotrophic Assay	Uterotrophic	Uterotropic	Uterine Weight	Uterus
Azoxystrobin	0	0	0	0	0
Flutamide	6	8	3	22	62
Butyl benzoate	6	8	0	5	7
Sodium tetrafluoroborate	0	0	0	0	0
Methyl parathion	0	0	0	2	2
13311-84-7 [rn]	3	5	2	17	50
134-20-3 [rn]	0	0	0	0	0

Abbreviation: rn = PubMed identifier for Chemical Abstract Service Registry Number

Numbers indicate the number of articles found. Each number is a hyperlink that takes the user to a PubMed results page for that particular keyword combination.

- To eliminate duplicate articles, search results were cross-referenced both within and between the different literature search engines.
- Where possible, each article was identified using its PubMed Identifier (PMID), a unique identifier developed, assigned, and maintained by PubMed.
- Each article that was not indexed by PubMed was assigned an arbitrary unique identifier (uID), for example NICEATM_01.
- Articles were saved as files named with their PMID/uID, allowing a direct link between a database entry and the file containing the data.

Development of the Database

- The literature review ontology was based on Rotroff (2013) and expanded to allow for comprehensive and standardized data entry across multiple users.
- The same information was collected for each chemical/protocol combination within each study:
  - Species of test animal (i.e., mouse or rat)
  - Ovariectomized vs. immature test animal
  - Test animal age at first dose
  - Use of positive and vehicle controls
  - Number of animals used in each treatment group
  - Treatment route, duration, and number of doses used
  - Time of necropsy after last dose
  - Phytoestrogen level in the rodent diet
  - Lowest effect level and direction of response (i.e., increase or decrease)

Data Review

Minimum Criteria for Guideline-Like Studies

- Two independent reviewers curated the data and scored the studies for adherence to minimum guideline criteria (**Table 2**). Discrepancies were resolved during a group review.
  - A score of 1 was assigned for each of the six minimum criteria that were met.
  - A score of 0 was assigned for each criterion that was not met.
  - Scores for the six minimum criteria were added. Studies with a score of 6 were considered guideline-like studies. (Note: This strategy may not represent the views of the EPA.)

Table 2. Assignment of Scores for Minimum Criteria

Score	Category	Criterion
1 or 0	Rodent model	Immature rats: Must be treated at 18 to 25 days of age Ovariectomized rats or mice: Surgery must be performed at 6 to 8 weeks of age with a 14-day recovery for rats and 7-day recovery for mice
1 or 0	Number of animals	Each test group and control should have a minimum of 3 animals (4 is preferred for negative results)
1 or 0	Route of exposure	Oral, subcutaneous, or intraperitoneal
1 or 0	Number of doses	At least 2 doses of test substances, with positive and negative controls
1 or 0	Treatment duration	At least 3 days
1 or 0	Time of necropsy	18 to 36 hours after the last treatment

- A sample of the database is shown in **Figure 3**.

Acknowledgements

The Intramural Research Program of the National Institute of Environmental Health Sciences (NIEHS) supported this poster. Technical support was provided by ILS under NIEHS contracts N01-ES 35504 and HHSN27320140003C.

The views expressed above do not necessarily represent the official positions of any Federal agency. Since the poster was written as part of the official duties of the authors, it can be freely copied.

A summary of NICEATM and ICCVAM activities at the Ninth World Congress is available on the National Toxicology Program website at http://ntp.niehs.nih.gov/go/41583.

References

A reference list for this poster is available at http://ntp.niehs.nih.gov/iccvam/meetings/9wc/ceger-edlitrev-refs.pdf